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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/004,494	Confirmation No. 9399
Applicant	:	Chang, Yung-Fu	
Filed	:	November 2, 2001	
Title	:	*Ehrlichia canis* genes and vaccines	
Art Unit	:	1632	
Examiner	:	Montanari, David A.	
Docket No.	:	1258-006 CIP	
Customer No.	:	20874	

DECLARATION OF YUNG-FU CHANG, D.V.M., Ph.D.
UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, YUNG-FU CHANG, D.V.M., Ph.D. do declare that:

1. I am the inventor of the invention disclosed and claimed in the above-identified patent application. A copy of my curriculum vitae is attached as Exhibit 1.

2. I am a Professor of Population Medicine & Diagnostic Sciences at the College of Veterinary Medicine of Cornell University. The Cornell Research Foundation, Inc., a not-for-profit affiliated corporation of Cornell University, is the assignee of the above-identified patent application.

3. I have read and understood the above-identified patent application and the Office Action dated December 12, 2006 ("Office Action") issued in connection therewith.

4. I understand that the above-identified application is a continuation-in-part application of prior application Serial No. 09/358,322, filed July 21, 1999 (now abandoned).

5. I understand that at pages 2-3 of the Office Action, claims 47 and 69 (Exhibit 2) were rejected under 35 U.S.C. 102(b), as being anticipated by Lewis *et al.* (1994, Sequence, organization, and evolution of the A+T region of Drosophila melanogaster mitochondrial DNA. Mol. Biol. Evol. 11: 523-538). The Examiner maintains that the DNA sequence disclosed in Lewis *et al.* shares stretches of homology that would encode some portion of a protein set forth in the sequences claimed in claims 47 and 69 (*i.e.*, SEQ ID NOs: 3, 5, 7, 9 or 11).

6. I am making this declaration to explain that using routine methods of analysis available in the art, I was unable to find any homology between the sequences disclosed Lewis *et al.* and any portion of the protein sequences claimed in claims 47 and 69.

7. Specifically, to determine whether there was any homology between the sequences disclosed Lewis *et al.* and any portion of the protein sequences claimed in claims 47 and 69, I used the National Center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov/) to identify the sequence disclosed in Lewis *et al.* as bases 14917-19517 of sequence NC_001709. See Exhibit 3, page 1 of the NCBI GenBank listing for sequence NC_001709, wherein the Lewis *et al.* reference is listed as "REFERENCE 2 (bases 14917 to 19517)."

8. SEQ ID NOs 3, 5, 7, 9 and 11 of the present invention are deposited in the NCBI GenBank database as accession number AF219120, "Ehrlichia canis cytochrome C oxidase assembly protein, protease A, and protease B genes, complete cds; and unknown gene" (Exhibit 4).

9. On March 9, 2007, I used the Lewis *et al.* sequence of bases 14917 to 19517 to perform a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/), using a translated query versus protein database search (BLASTX 2.2.16, which version was designated as the "Mar-11-2007" version). I obtained the results shown in Exhibit 5, which indicate no homology between the queried sequence (translation of the Lewis *et al.* sequence) and any protein sequence in the NCBI protein database, including SEQ ID NOs 3, 5, 7, 9 and 11 (deposited as accession number AF219120).

10. According to my analysis, I conclude that the translation of the sequence disclosed in the Lewis *et al.* reference does not disclose the amino acid sequences SEQ ID NOs 3, 5, 7, 9 or 11 as claimed in claims 47 and 69, nor does it disclose homologous sequences. Moreover, an ordinarily skilled artisan would not understand Lewis *et al.* as disclosing a sequence of bases that, when translated, provide sequence(s) that share homology with the amino acid sequences claimed in claims 47 and 69 or homologous sequences, and such a person could not have combined the Lewis *et al.* reference's description of bases 14917 to 19517 of the *Drosophila melanogaster* mitochondrion genome with his own knowledge to make the claimed invention.

11. The results set forth in Exhibit 5, as described above, therefore rebut the rejection at pages 2-3 of the Office Action that the claimed invention is anticipated by Lewis *et al.*

12. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 4-12-2007

YUNG-FU CHANG, D.V.M., Ph.D.

Attachments:

Exhibit 1: Curriculum Vitae of Yung-Fu Chang, D.V.M., Ph.D.

Exhibit 2: Listing of Claims as Presently Amended in the Accompanying Amendment

Exhibit 3: NCBI GenBank Listing for Sequence NC_001709

Exhibit 4: NCBI GenBank Listing for Sequence AF219120

Exhibit 5: BLASTX Analysis of Lewis *et al.* Sequence

EXHIBIT 1

Curriculum Vitae of Yung-Fu Chang, D.V.M., Ph.D.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Yung-Fu Chang	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
National Pintung University of Science and Technology	DVM	1974	Veterinary Medicine
University of Idaho	MS	1981	Immunology/Pathology
Texas A&M University, College Vet. Med.	PhD	1984	Microbiology
Texas A&M University, College Med.	Post-doc	1989	Molecular Biology/Genetics

A. RESEARCH AND PROFESSIONAL EXPERIENCE:

1974-1979 Assistant Pathologist, Veterinary Pathology Division, Taiwan Provincial Research Institute for Animal Health, Taiwan

1984-1985 Research Associate, Medical Biochemistry and Genetics, College of Medicine, Texas A&M University, College Station, Texas

1986-1989 Assistant Research Scientist, Medical Biochemistry and Genetics, College of Medicine, Texas A&M University, College Station, Texas

1989-present Assistant/Associate/Full Professor, Department of Population Medicine & Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York.

2003(Jan. to July). Visiting Professor, Department of Infectious disease and Medicine, College of Medicine, Stanford University. Stanford, California.

PROFESSIONAL SOCIETIES: Society of Sigma Xi, American Society for Microbiology, The Society of PHi Zeta, American Association for the Advancement of Science.

RESEARCH INTERESTS: Molecular biology of infectious disease; DNA and recombinant subunit vaccine development; molecular basis of bacterial pathogenesis; immunopathology of infectious disease; DNA probes development; host defense mechanisms; Microarray for diagnosis & pathogenesis.

B. Selected peer-reviewed publications (in chronological order): (Selected papers out of

95)

Chang, Y. F., R. Young., D. Post, and D. K. Struck. 1987. Identification and characterization of the *Pasteurella haemolytica* leukotoxin. Infect. Immun. 55: 2348-2354.

Chang, Y. F., R. Young, T. L. Moulds, and D. K. Struck. 1989. Secretion of the *Pasteurella* leukotoxin by *E. coli*. FEMS Microbial. Let. 60: 169-174.

Chang, Y. F., R. Young, and D. K. Struck. 1989. Cloning and characterization of a hemolysin gene from *Actinobacillus (Haemophilus) pleuropneumoniae* DNA &Cell biology: 8: 635-647.

Cruz, W.T., R. Young, **Y. F. Chang**, and D.K. Struck. 1990. Deletion analysis resolves cell-binding and lytic domains of the *Pasteurella* leukotoxin. Mol. Microbiol. 4: 1933-1939.

Chang, Y. F., R. Young, and D.K. Struck. 1991. The *Actinobacillus pleuropneumoniae* determinant: unlinked *appCA* and *appBD* loci flanked by pseudogenes. J. Bacteriol. 173: 5151-5158.

McWhinney, D.R., **Y. F. Chang**, R.Young, and D. K.Struck. 1992 Separable domains define target cell specificities of the RTX hemolysin from *Actinobacillus pleuropneumoniae*. J. Bacteriol. 174: 291-297.

Appel, M. J.G., S. Allan, R. H. Jacobson, T.L. Lauderdale, **Y. F. Chang**, S. J. Shin, J. Thomford, R. Todhunter, and B. A. Summers. 1993. Experimental Lyme disease in dogs produced arthritis and persistent infection. J. Infect. Dis. 167: 651-664.

Chang, Y. F., D. P. Ma, J. Shi, and M. M. Chengappa. 1993. Molecular characterization of a leukotoxin gene from a *Pasteurella haemolytica*-like organism, encoding a new member of RTX family. Infect. Immun. 61: 2089-2095.

Chang, Y. F., J. Shi, D. P. Ma, S. J. Shin, and D. H. Lein. 1993. Molecular analysis of the *Actinobacillus pleuropneumoniae* RTX toxin-III gene cluster. DNA. Cell Biol. 12: 351-362.

Chang, Y. F., T. L. Lauderdale, W. Y. Lee, S. J. Shin, R. H. Jacobson, M. J. Appel, and D. H. Lein. 1993. Expression and secretion of outer surface protein (OspA) of *Borrelia burgdorferi* from *E. coli*. FEMS Microbiol. Lett. 109: 297-302.

Frey, J., J. T. Bosse, **Y. F. Chang**, J.M. Cullen, B. Fenwick, G.F. Gerlach, D. Gygi, F. Haesebrouck, T. J. Inzana, R. Jansen, E. M. Kamp, J. Macdonald, J. I. MacInnes, K.R. Mittal, J. Nicolet, A.N. Rycroft, R.P.A.M. Segers, M.A. Smits, E. Stenbaek, D.K. Struck, J. F. Van Den Bosch, P. J. Wilson and R. Young. 1993. *Actinobacillus pleuropneumoniae* RTX-toxins: Uniform designation of hemolysins, cytolysins, pleurotoxin and their genes. J. Gen. Microbiol. 139: 1723-1728.

Chang, Y. F., M. J. Appel, R. H. Jacobson, S. J. Shin, P. Harpending, R. Straubinger, L. A. Patrican, H. Mohammed, and B. A. Summers. 1995. Recombinant OspA protects dogs against infection and disease caused by *Borrelia burgdorferi*. Infect. Immun. 63: 3543-3549.

Straubinger, R. K., **Y. F. Chang**, R. H. Jacobson, and M. J. G. Appel. 1995. Protection against Lyme disease: Sera from vaccinated dogs, but not from tick infected dogs, inhibit the *in vitro* growth of *Borrelia burgdorferi*. J. Clin. Microbiol. 33: 2745-2751.

Chang, Y. F., R. Straubinger, R. H. Jacobson, J. B. Kim, T. J. Kim, D. Kim, S. J. Shin, and M. J. G. Appel. 1996. Dissemination of *Borrelia burdorferi* after experimental infection in dogs. J. Spiro. Tick-Borne Dis. 3: 80-86.

Straubinger, R. K., A. F. Straubinger, L. Harter, R. H. Jacobson, **Y. F. Chang**, B. A. Summers, H. N. Erb, and M. J. G. Appel. 1997. *Borrelia burgdorferi* migration and

proliferation causes up-regulation of interleukin-8 in synovial membranes of experimentally infected dogs. Infect. Immun. 65: 1273-1285.

McDonough P. L., R. H. Jacobson, J. F. Timoney, A. Mutalib, D. C. Kradel, **Y. F. Chang**, S. J. Shin, D. H. Lein, S. Trock, and K. Wheeler. 1998. Interpretations of antibody responses to *Salmonella enterica* serotype Enteritidis gm flagellin in poultry flocks are enhanced by a Kinetics-based enzyme-linked immunosorbent assay. Clin. Diagn. Lab. Immunol. 5: 550-555.

Chang, Y. F., V. Novosel, S. P. McDonough, R. H. Jacobson, C. F. Chang, T. Divers, F. W. Quimby, S. Shin, and D. H. Lein. 1999. Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer surface protein A (OspA) in horses. Vaccine 18: 540-548.

Chang, Y. F., S. P. McDonough, K.S. Shin, C. F. Chang, and T. Divers. 2000. Human granulocytic ehrlichiosis agent (HGE) infection in a pony vaccinated with recombinant OspA vaccine and challenged by exposure to naturally infected ticks. Clin. Diag. Lab. Immunol. 7: 68-71.

Simpson, K.W., D. Strauss-Ayali, E. Scanziani, R. K. Straubinger, P. L. McDonough, A.F. Straubinger, **Y. F. Chang**, C. Domeneghini, N. Arebi, and J. Calm. 2000. *Helicobacter felis* infection is associated with lymphpoid follicular hyperplasia and mild gastritis but normal gastric secretory function in cats. Infect. Immun. 68: 779-790.

Simpson, K.W., D. Strauss-Ayali, E. Scanziani, R. K. Straubinger, P. L. McDonough, A. F. Straubinger, **Y. F. Chang**, M. Esteves, J. G. Fox, C. Domeneghini, N. Arebi, and J. Calam. 2001. Gastric secretory function in cats with *Helicobacter pylori* infection. Helicobacter 6: 1-14.

Raghavan, P. U. M., **Y. F. Chang**, S. S. D. Jusuf, S. Artiushin, J. F. Timoney, S. P. McDonough, S. C. Barr, T. J. Divers, P. McDonough, K. W. Simpson, and H. Mohammed. 2002. Cloning and molecular characterization of an immunogenic LigA of Leptospira interrogans. Infect. Immun. 70:5924-5930.

Dheenadhayalan, V., K. S. Shin, C. F.Chang, C. D. Chang, S. J. Wang, S. P. McDonough, P. L. McDonough, S. Shin, A. Torres, and **Y. F. Chang**. 2002. Cloning and characterization of the genes coding for antigen 85A, 85B and 85C of *Mycobacterium avium* subsp. paratuberculosis. DNA Seq. 13:287-294.

Teng, C.H., R. U.M. Palaniappan, and **Y. F. Chang**. 2003. Cloning and characterization of an *Ehrlichia canis* gene encoding a protein localized to the morula membrane. Infect. Immun. 71:2218-2225.

Hsu, Y.M., N. Chin, C. F. Chang, **Y. F. Chang**. 2003. Cloning and characterization of the *Actinobacillus pleuropneumoniae fur* gene and its role in regulation of ApxI and AFUABC expression. DNA seq. 14:169-181.

Santos, L. R. D., S. M. Barrouin-Melo, **Y. F. Chang**, J. Olson, S. P. McDonough, F. Quimby, W. L. C. D. Santos, L. C. Pontes-de-Carvalho, G. G. Oliveria. 2003. Recombinant single-chain interleukin-12 induces interferon mRNA expression in peripheral blood mononuclear cells of dogs with visceral leishmaniasis. Vet. Immun. Immunopathol. 98:43-48.

Palaniappan, R. U., **Y. F. Chang**, F. Hassan, S.P. McDonough, M. Pough, S.C. Barr, K. W. Simpson, H. O. Mohammed, S. Shin, P. McDonough, R. Zuerner, J. Qu, & B. Roe. 2004. Expression of leptospiral immunoglobulin-like protein from *Leptospiral interrogans* and evaluation of its diagnostic potential in kinetic enzyme linked

immunosorbent assay. J. Med. Microbiol. 53: 975-84.

Wang, Z., Z. Yuan, M. Matsumoto, U. R. Hengge and **Y. F. Chang**. 2005. Immune responses with DNA vaccines encoded different gene fragments of severe acute respiratory syndrome coronavirus in BALB/c mice. BioChem. BioPhy. Res. Com. 327:130-135.

Palaniappan, R. U., **Y. F. Chang**, C. F. Chang, M. J. Pan, C. W. Yang, P. Harpending, S. P. McDonough, E. Dubovi, J. Qu, B. Roe and T. Divers 2005. Evaluation of *lig*-based conventional and real time PCR for the detection of pathogenic leptospires. Mol. and Cell. Probes. 19:111-117.

Hsieh, W.J., **Y.F. Chang,** C. S. Chen, and M. J. Pan. 2005. Omp52 is a growth-phase-regulated outer membrane protein of *Leptospira santarosai* serovar Shermani. FEMS Microbiol. Let. 243:339-345.

Shin, S.J., C.F. Chang, C. C. Chang, S. P. McDonough, B. Thompson, H.S. Yoo, and **Y. F. Chang**. 2005. In vitro cellular immune response to recombinant antigens of *Mycobacterium avium* subsp. *paratuberculosis*. Infect. Immun. 5074-5085.

Ku, Y.W., S. P. McDonough, R.U.M. Palaniappan, C. F. Chang, **Y. F. Chang.** 2005. Identification and characterization of *in vivo* attenuated mutants of *Salmonella enterica* serovar Choleraesuis using signature-tagged mutagenesis in a pig infection model. Infect. Immun. 73:8194-8203.

Raghavan, P. U. M., S. P. McDonough, T. J. Divers, C. S. Chen, M.J. Pan, M. Matsumoto and **Y. F. Chang**. 2006. Immunoprotection of recombinant leptospiral immunoglobulin like A protein (LigA) against *Leptospira interrogans* serovar Pomona infection. Infect. Immun. 74:1745-1750.

Palaniappan, R. U., Y. Zhang, D. Chiu, A. Torres, C. DebRoy, T. S. Whittam and **Y. F. Chang**. 2006. Differentiation of *Escherichia coli* pathotypes by oligonucleotide spotted array. J. Clin. Microbiol. 44:1495-1501.

Palaniappan, R. U., S. Ramanujam and **Y. F. Chang**. 2006. Leptospirosis: Pathogenesis, Immunity, and Diagnosis. Current Opinion Infect. Dis. In press.

Palaniappan, R. U., D. Chiu, H. He, P. Harpending and **Y. F. Chang**. 2006. Identification of immunogenic proteins from genome of *Leptospira interrogans*. Clin. Vaccine & immunol. Submitted.

Chang, Y.F., C. S. Chen, R. U.M. Palaniappan, S. P. McDonough, W. Yang, M. J. Pan, and C. F. Chang. Immunogenicity and protection of the rtecombinant leptospiral immunogenic proteins as vaccine candidates. Infect. Immun. Submitted.

Faisal, S.M., W. Yan, C. S. Chen, R. U.M. Palaniappan, S. P. McDonough, and **Y. F. Chang**. Evaluation of protective immunity of Leptospira immunoglobulin like protein A (LigA) DNA vaccine against challenge in hamsters. Infect. Immun. Submitted.

Lin, Y.P. and **Y. F. Chang.** Molecular characterization of LigB, a Fibronectin-Binding Protein of *Leptospira.* Infect. Immun. Submitted.

Kathaperumal K., S. U. Park, S. P. McDonough, S. Stehman, B. Akey, J. Huntley, S. Wong, L. H. Chen, C.-F. Chang and **Y. F. Chang.** Vaccination with recombinant *Mycobacterium avium* subsp.*paratuberculosis* proteins induces differential immune responses and protects calves against infection by oral challenge. Infect. Immun. Submitted.

Chen, L. H., K. Kathaperumal, C. J. Huang, S. P. McDonough, S. Stehman, B. Akey, J. Huntley, C.F. Chang and **Y. F. Chang**. Immune responses in mice to *Mycobacterium avium* subsp. *paratuberculosis* following vaccination with a novel 74F recombinant polyprotien. Infect. Immun. Submitted.

Park, S. U., K. Kathaperumal, S. P. McDonough, S. Stehman, B. Akey, J. Huntley, and **Y. F. Chang**. Immunization with DNA Vaccine against Johne's disease in an experimental mouse model. Infect. Immun. Submitted.

Scaria, J., B. Raveendran, and **Y. F. Chang**. Horizontal gene transfer is a major force influencing codon usage variation in *M. avium ssp. paratuberculosis* and *M. smegmatis.* BMC Microbiol. Submitted.

Cui, Y., D. Luo, P. L. McDonough and **Y. F.Chang**. Simultaneous Detection of *Salmonella* spp. and *Campylobacter* spp. with Quantum Dots. Anal. Bioanal. Chem. Submitted.

Kumanan, V. S. R. Nugen, A. J. Baeumner, and **Y. F. Chang**. A rapid biosensor assay for the detection *Mycobacterium avium* subsp. *paratuberculosis* from fecal samples. Anal. Bioanal. Chem. Submitted.

C. Research Support

Current Research Support

Chang 7/01/06-06/30/07
CAT
Bacterial protein microarays for identification of new potential diagnostic markers and vaccine candidate for *Leptospira* spp. infection.
This grant focuses on the development of a serologic test/vaccine candidates for animal leptospirosis.

Chang 11/1/03-10/31/07
BRDC
Paratuberculosis: Novel DNA vaccine with single chain bovine IL-12 adjuvant.
This grant focuses on the development of a DNA vaccine against bovine paratuberculosis.

Chang 11/1/03-10/31/07
BRDC
Identification of *L. borgpetersenii* serovar Harjo virulence factors and vaccine development.
This grant focuses on the identification of leptospiral virulence factors and the development of a recombinant vaccine against animal leptospirosis.

Chang 4/1/03-3/31/07
NIH (N01-A1-30054;ZC002-07)
Molecular diagnosis (microarray) of bacterial pathogens.
This grant focuses on the development a microarray test for food and water borne pathogens.

Chang 10/1/04-9/30/07.
CUAES Hatch project.
Biosensor for rapid detection of *Mycobacterium avium* subsp. *paratuberculosis.*
This grant focuses on the development of a biosensor detection for Johne's disease.

Chang 10/1/05-9/30/08.

CUAES Hatch project.
Development a live vaccine against bovine salmonelosis.
This grant focuses on the development of an attenuated *Salmonella* vaccine for cattle.

Chang 1/1/05-12-30/07.
Zweig.
Virulence factors of serovar Pomona and vaccine development.
This grant focuses on the identification of virulence factors of serovar Pomona.

Chang (Co-PI), Luo (PI) 1/1/06-12/30/07
NY State (NYSTAR)
DNA-based Nanobarcode Technology for Molecular Detections in Biology, Veterinary and Anti-bioterrorism Fields
This is a translational grant aiming at research and development for the commercialization of DNA Nanobarcode Technology.

Chang 12/1/03-11/30/09
NIH (N01-A1-30054;ZC007-09)
C. difficile: A comparative genomics and transcriptome study.
This grant focuses on a comparative genomics and transcriptome study of *C. difficile*.

EXHIBIT 2

Listing of Claims as Presently Amended in the Accompanying Amendment

1 – 46 (canceled).

47 (currently amended). An isolated recombinant DNA comprising a DNA selected from the group consisting of

a) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3 wherein the protein elicits an immune response against *E. canis*;

b) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5 wherein the protein elicits an immune response against *E. canis*;

c) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ.ID. NO. 7 wherein the protein elicits an immune response against *E. canis*;

d) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9 wherein the protein elicits an immune response against *E. canis*; and

e) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11 wherein the protein elicits an immune response against *E. canis*.

48-68 (canceled).

69 (currently amended). A vector capable of expressing [[a]] an isolated recombinant DNA comprising the isolated recombinant DNA inserted into the vector such that a recombinant protein is expressed when the vector is provided in an appropriate host wherein the isolated recombinant DNA is selected from the group consisting of:

a) SEQ. ID. NO. 2, wherein SEQ. ID. NO. 2 encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3 and wherein the protein elicits an immune response against *E. canis*;

b) SEQ. ID. NO. 4, wherein SEQ. ID. NO. 4 encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5 and wherein the protein elicits an immune response against *E. canis*;

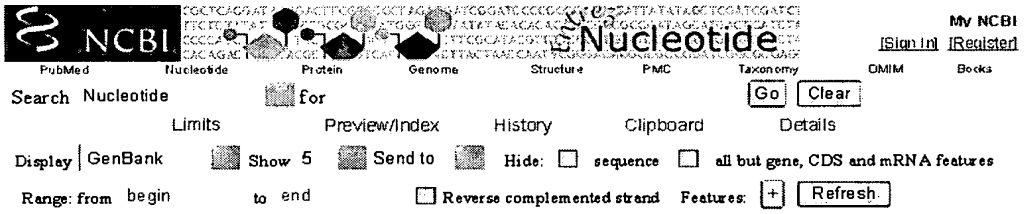
c) SEQ. ID. NO. 6, wherein SEQ. ID. NO. 6 encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7 and wherein the protein elicits an immune response against *E. canis*;

d) SEQ. ID. NO. 8, wherein SEQ. ID. NO. 8 encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9 and wherein the protein elicits an immune response against *E. canis*; and

e) SEQ. ID. NO. 10 wherein SEQ. ID. NO. 10 encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11 and wherein the protein elicits an immune response against *E. canis*.

EXHIBIT 3

NCBI GenBank Listing for Sequence NC_001709

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LOCUS       NC_001709              19517 bp    DNA     circular INV 10-AUG-2005
DEFINITION  Drosophila melanogaster mitochondrion, complete genome.
ACCESSION   NC_001709
VERSION     NC_001709.1  GI:5835233
PROJECT     GenomeProject:164
KEYWORDS    .
SOURCE      mitochondrion Drosophila melanogaster (fruit fly)
  ORGANISM  Drosophila melanogaster
            Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta; Pterygota;
            Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha;
            Ephydroidea; Drosophilidae; Drosophila.
REFERENCE   1  (bases 1 to 408; 13319 to 19517)
  AUTHORS   Lewis,D.L., Farr,C.L. and Kaguni,L.S.
  TITLE     Drosophila melanogaster mitochondrial DNA: completion of the
            nucleotide sequence and evolutionary comparisons
  JOURNAL   Insect Mol. Biol. 4 (4), 263-278 (1995)
   PUBMED   8825764
REFERENCE   2  (bases 14917 to 19517)
  AUTHORS   Lewis,D.L., Farr,C.L., Farquhar,A.L. and Kaguni,L.S.
  TITLE     Sequence, organization, and evolution of the A+T region of
            Drosophila melanogaster mitochondrial DNA
  JOURNAL   Mol. Biol. Evol. 11 (3), 523-538 (1994)
   PUBMED   8015445
REFERENCE   3  (bases 14215 to 14512)
  AUTHORS   Ballard,J.W., Olsen,G.J., Faith,D.P., Odgers,W.A., Rowell,D.M. and
            Atkinson,P.W.
  TITLE     Evidence from 12S ribosomal RNA sequences that onychophorans are
            modified arthropods
  JOURNAL   Science 258 (5086), 1345-1348 (1992)
   PUBMED   1455227
REFERENCE   4  (bases 441 to 2967)
  AUTHORS   Satta,Y. and Takahata,N.
  TITLE     Evolution of Drosophila mitochondrial DNA and the history of the
            melanogaster subgroup
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 87 (24), 9558-9562 (1990)
   PUBMED   2124697
REFERENCE   5  (bases 5268 to 13619)
  AUTHORS   Garesse,R.
  TITLE     Drosophila melanogaster mitochondrial DNA: gene organization and
            evolutionary considerations
  JOURNAL   Genetics 118 (4), 649-663 (1988)
   PUBMED   3130291
REFERENCE   6  (bases 804 to 1778)
  AUTHORS   Satta,Y., Ishiwa,H. and Chigusa,S.I.
  TITLE     Analysis of nucleotide substitutions of mitochondrial DNAs in
            Drosophila melanogaster and its sibling species
  JOURNAL   Mol. Biol. Evol. 4 (6), 638-650 (1987)
   PUBMED   2832697
REFERENCE   7  (bases 404 to 5272)
  AUTHORS   de Bruijn,M.H.
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  TITLE     Drosophila melanogaster mitochondrial DNA, a novel organization and
            genetic code
  JOURNAL   Nature 304 (5923), 234-241 (1983)
   PUBMED   6408489
REFERENCE   8  (bases 5269 to 5695)
  AUTHORS   Clary,D.O., Wahleithner,J.A. and Wolstenholme,D.R.
  TITLE     Transfer RNA genes in Drosophila mitochondrial DNA: related 5'
            flanking sequences and comparisons to mammalian mitochondrial tRNA
            genes
  JOURNAL   Nucleic Acids Res. 11 (8), 2411-2425 (1983)
   PUBMED   6304652
REFERENCE   9  (bases 12511 to 12682)
  AUTHORS   Clary,D.O., Goddard,J.M., Martin,S.C., Fauron,C.M. and
            Wolstenholme,D.R.
  TITLE     Drosophila mitochondrial DNA: a novel gene order
  JOURNAL   Nucleic Acids Res. 10 (21), 6619-6637 (1982)
   PUBMED   6294611
REFERENCE   10 (bases 1 to 19517)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (08-SEP-1999) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   11 (bases 1 to 19517)
  AUTHORS   Lewis,D.L., Farr,C.L. and Kaguni,L.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (03-OCT-1995) Laurie S. Kaguni, Biochemistry Department,
            Michigan State University, East Lansing, MI 48824-1319, USA
COMMENT     REVIEWED REFSEQ: This record has been curated by NCBI staff. The
            reference sequence was derived from U37541.
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                NFITTVINMRSTGISLDRMPLFVWSVVITALLLLLSLPVLAGAITMLLTDRNLNTSFF
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                AILWALGFVFLFTVGGLTGVVLANSSVDIILHDTYYVVAHFHYVLSMGAVFAIMAGFI
                HWYPLFTGLTLNNKWLKSHFIIMFIGVNLTFFPQHFLGLAGMPRRYSDYPDAYTTWNI
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                /db_xref="GeneID:192470"
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                QWYWSYEYSDFNNIEFDSYMIPTNELMTDGFRLLDVDNRVVLPMNSQIRLLVTAADVI
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                TQGLFFTVLLGIYFTILQAYEYIEAPFTIADSIYGSTFFMATGFHGIHVLIGTTFLLV
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                     VMTFLFDWMSLLFMSFVLMISSLVIFYSKEYMMNDNHINRFIMLVLMFVLSMMLLIIS
                     PNLISILLGWDGLGLVSYCLVIYFQNIKSYNAGMLTALSNRIGDVALLLSIAWMLNYG
                     SWNYIPYLEIMQNEFEMLMIGSLVMLAAMTKSAQIPFSSWLPAAMAAPTPVSALVHSS
                     TLVTAGVYLLIRFNIILSTSWLGQLMLLLSGLTMFMAGLGANFEFDLKKIIALSTLSQ
                     LGLMMSILSMGFLKLAMFHLLTHALFKALLFMCAGAIIHNMNNSQDIRLMGGLSIGIH
                     MPLTSACFNVSNLALCGMPFLAGFYSKDMILEIVSISNVNMFSFFLYYFSTGLTVSYS
                     FRLVYYSMTGDLNCGSLNMLNDESWIMLRGMMGLLIMSIIGGSMLNWLIFPFPYMICL
                     PIYMKLLTLFVCIVGGLFGYLISLSNLFFLNKSLFMYNLSTFLGSMWFMPYISTYGMI
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                     VMNKIGSMNFYLMNNFMFNYDLLYFCLLCAFLVKMPMFLVYLWLPKAHVEAPVSGSMI
                     LAGIMLKLGGYGMLRVISFIQLMNLKYSFVWISISLVGGVLVSLVCLRQTDLKALIAY
                     SSVAHMGIVLSGLLTMTYWGLCGSYTLMIAHGLCSSGLFCLANVSYERLGSRSMLINK
                     GLLNFMPSMTLWWFLLSSANMAAPPTLNLLGEISLLNSIVSWSWISMILLSFLSFFSA
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                     /db_xref="GeneID:192477"
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gene                 9837..9902
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EXHIBIT 4

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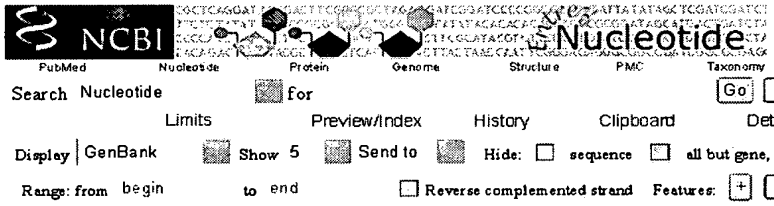

1: AF219120 Reports Ehrlichia canis c...[gi:27462093] Links

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ACCESSION   AF219120
VERSION     AF219120.1  GI:27462093
KEYWORDS    .
SOURCE      Ehrlichia canis
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REFERENCE   1  (bases 1 to 5300)
  AUTHORS   Teng,C.H., Palaniappan,R.U.M. and Chang,Y.F.
  TITLE     Cloning and Characterization of an Ehrlichia canis Gene Encoding a
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  JOURNAL   Infect. Immun. 71 (4), 2218-2225 (2003)
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  AUTHORS   Chang,Y.-F. and Teng,C.-T.
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     2041 aagtaaaagc acatttaact tatgcatttg acggactaac tttcatatca tatttttatg
     2101 gcatgcatct aatactagga gtaccgctat cagaaatcag taatatttac gataccatag
     2161 acaaagtaag tatccaagat gttaactccg ctatggaaaa tatctttcaa aacaatataa
     2221 gattaaccgg gcatttatta cctaatggag aatagttatg agaaacatat tgtgttacac
     2281 attaatattg attttctttt cattcaatac atatgcaaat gatctcaata ttaacataaa
```

```
2341 agaagctaca actaaaaata aaatacacta tctatatgtt gaacatcata acctaccaac
2401 aatttcctta aaatttgcat tcaagaaagc aggatacgct tatgatgcct ttgataagca
2461 aggacttgca tactttacat caaaaatatt aaacgaagga tcaaaaaaca actatgctct
2521 cagttttgca caacaattag aaggcaaagg tatagactta aaatttgata tagacctaga
2581 caatttttat atatcattaa aaaccttatc agaaaacttt gaagaagccc tagttttact
2641 cagtgattgc atattcaaca ccgtcacaga tcaagaaata ttcaatagaa taatagcaga
2701 acagattgca catgttaaat cattatattc tgctcctgaa tttatagcta caacagaaat
2761 gaatcacgct atattcaaag ggcacccata ttctaacaaa gtttacggga cattaaatac
2821 aatcaataat atcaaccagg aagacgttgc attatatata aaaaatagtt ttgacaagga
2881 acaaatcgtt atcagcgcag caggagatgt agatccaaca cagctatcaa atttactaga
2941 taaatatatt ctttccaaat tgccatctgg taataacaaa aataccatac cagatacgac
3001 tgttaatagc gaagacacat tattatatgt acagagagat gtaccacaaa gtgtcataat
3061 gtttgctaca gacacagtac catatcacag caaagactat catgcatcaa acttgttcaa
3121 tactatgcta ggcggattaa gtctcaattc aatattaatg atagaattaa gagacaagtt
3181 aggattaaca taccatagta gcagttcact atctaacatg aatcatagta atgtgctatt
3241 tggtacaata ttcactgata atacgacagt aacaaaatgt atatccgtct taacagatat
3301 tatagagcac attaaaaagt atggagttga tgaagacact tttgcaattg caaaatctag
3361 tattaccaac tcttttattt tatctatgtt aaataacaat aatgttagtg agatattgtt
3421 aagcttacaa ttacacgatc tagatccgag ttatattaat aaatacaatt cttactacaa
3481 agcaataaca atagaagaag taaataaaat tgccaagaaa attttatcta atgaattagt
3541 aataattgaa gtaggaaaaa acaataacat aaatggcaaa caaatagatg ctaaaaaaca
3601 cataccttgg ttaagtatac aggttattgt atttactaca agtattctat taggttgtat
3661 taagtaagta taagtagctt caatcaaata aaaaaacatt aaccaaagtg ttagctctac
3721 cggagaagct tattataagc ttttaacctg ggataatatg aagttttgct aatgttaagc
3781 aaaaaattag taatcacaat atcaaatttt ctttacagga ttatattgtg acctaccata
3841 acaacttata tttagaaaat gacaacagat acacacatca ataaattatc actacaattc
3901 aattaataaa acaatgagta tttttactta attatttaat tttatttttt aaaataaaat
3961 tacaatttta cttactcaat aaaagcagtt atactaccaa gtattggatg gtattaatcg
4021 gagcaattac tacttaatag tatagctgtt gacaagccgc aatctgcggt tcttgacaaa
4081 ataatactaa tcagttaaaa ttttgaagtg tttcaccata atggtattat ttatgaaagc
4141 tcatagcaca agtatacgga actttcagcc tttagaaaga gctgctataa tcattgcagt
4201 gttaggttta gctgcattct tgtttgctgc tgctgcctgc agtgatcgtt tccaaagatt
4261 gcaattaaca aatccatttg taatagcagg aatggttggc cttgcagttc ttttagttgc
4321 ttccttaaca gcagcattaa gtatatgctt aactaaaagt aagcaagtca cacaacatgc
4381 tattagacat cgctttggat acgagtcaag cacttcttct tctgtactgc ttgcaatatc
4441 aataatttct ttattacttg ctgcagcatt ttgtggaaag ataatgggta atgacaaccc
4501 agatctattc tttagcaaga tgcaagaact ctccaatcca cttgttgttg cagctattgt
4561 agccgtttct gttttcctac tctcattcgt aatgtatgct gcaaagaaca ttataagtcc
4621 agataaacaa actcacgtta ttatattatc taatcaacaa actatagaag aagcaaaagt
4681 agatcaagga atgaatattt tgtcagcagt actcccagca gctggcattg acatcatgac
4741 tatagcttct tgtgacattt tagcagtgag cagccgggga tcctctcagc atcaatagat
4801 ttatgtttta gcctgtattc acctttttat taggtgttgt atcgtttctt tatataagtg
4861 tgttatatta tataaaacat ctaggagtta cagttaattt gtttcatgtg gttattactc
4921 tttgccatta ttattactat acctaaaaat ataaaagaat ccgccaggtt gaatacaggc
4981 caatgtaagt tattgatata aaaatctata aaatcataga cagcaccata tcttattcta
5041 tctatgatat ttcctattga ccccccaata atgattacaa gaggtaatct ataatgtggc
5101 tgtactataa ataagtagcs taaaacacaa gtaatcaaaa tcgagatact acaaaaaaca
5161 acattactat attcaaagtt atttaatata ccaaaactaa ttccagcatt ccacactgta
5221 gtaaagcgca agaagcttaa tatctctatt acacctttat ctcctatcaa atttactaca
5281 taccatttac ttacctgatc
//
```

EXHIBIT 5

BLASTX Analysis of Lewis *et al.* Sequence

BLASTX 2.2.16 [Mar-11-2007]
<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed&cmd=Retrieve&list_uids=9254694&dopt=Citation>Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman
(1997), "Gapped BLAST and PSI-BLAST: a new generation of
protein database search programs", Nucleic Acids Res. 25:3389-3402.

RID: 1173814264-27220-33197384427.BLASTQ2

Database: All non-redundant GenBank CDS
translations+PDB+SwissProt+PIR+PRF excluding environmental samples
4,736,044 sequences; 1,634,373,987 total letters

If you have any problems or questions with the results of this search
please refer to the
<http://www.ncbi.nlm.nih.gov/blast/blast_FAQs.html>BLAST FAQs
<http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Get&RID=1173814264-27220-33197384427.BLASTQ2&FORMAT_OBJECT=TaxBlast&NCBI_GI=on&DESCRIPTIONS=100&ALIGNMENTS=50&FORMAT_BLOCK_ON_RESPAGE=Top&MASK_COLOR=1&MASK_CHAR=2>Taxonomy
reports

Query=
Length=4624

No significant similarity found. For reasons why,
<http://www.ncbi.nlm.nih.gov/blast/blast_FAQs.html#no%20hits>click here.

Database: All non-redundant GenBank CDS
translations+PDB+SwissProt+PIR+PRF excluding
environmental samples
Posted date: Mar 12, 2007 5:53 PM
Number of letters in database: 1,634,373,987

Number of sequences in database: 4,736,044
Lambda K H
0.318 0.134 0.401
Gapped
Lambda K H
0.267 0.0410 0.140
Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Sequences: 4736044
Number of Hits to DB: 78697997
Number of extensions: 633651
Number of successful extensions: 1607
Number of sequences better than 10: 0
Number of HSP's better than 10 without gapping: 0
Number of HSP's gapped: 1605
Number of HSP's successfully gapped: 0
Length of query: 4624
Length of database: 1634373987
Length adjustment: 145
Effective length of query: 4479
Effective length of database: 947647607
Effective search space: 1322916059372
Effective search space used: 1322916059372
T: 12
A: 40
X1: 16 (7.3 bits)
X2: 38 (14.6 bits)
X3: 64 (24.7 bits)
S1: 41 (20.4 bits)
S2: 84 (37.0 bits)